transport protein are suitable for use in the present invention.

Page 7, first full paragraph:

A suitable substantially identical protein is a protein having an amino acid sequence that is generally at least 90% identical to the amino acid sequence of human TREK-1 (SEQ ID NO:2). More preferably, the protein is at least 95% identical to SEQ ID NO:2. Most preferably, the amino acid sequence is at least 99% identical to SEQ ID NO:~~1~~2.

Paragraph bridging Pages 7 and 8:

The coding sequence of the potassium transport protein may be inserted between the noncoding sequences 5' and 3' of a *Xenopus laevis* protein (such as globin) in an appropriate vector, such as pEXO. The construct is introduced into an appropriate cell type to replicate the vector and/or to transcribe RNA. Alternatively, the vector may be used as a template for ~~in vitro~~*in vitro* transcription. A complementary RNA (cRNA) is transcribed and injected into a cell, such as a *Xenopus* oocyte. Such a procedure may be performed in a 0.3 ml perfusion chamber, wherein single oocytes are impaled on two standard glass microelectrodes (0.5-2.0 MW) charged with 3 M KCl and maintained under voltage clamp with a Dagan TEV200 amplifier~~;~~. The bath solution contains 98 mM KCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES at pH 7.4 with KOH.

Page 8, first full paragraph:

Alternatively, functional expression of the potassium channel may be accomplished by transfection of insect cells, such as *Spodoptera frugiperda* (Sf9) cells. Briefly a suitable vector, such as pVL1392 may be used and the coding sequence for the potassium transport protein may be inserted into the vector in-frame so that expression of the potassium transport protein may be expressed. The coding sequence for the potassium transport protein may be obtained by any convenient method, such as by PCR or by digesting a plasmid containing the potassium transport protein coding sequence with appropriate restriction endonuclease(s) for subsequent ligation into the pVL1392 vector. Similarly, the amplified product of the

PCR may be digested with restriction enzymes and ligated into the vector. Transfection of ~~SF9~~Sf9 cells may be performed by the manufacturer's protocol (Pharmingen).

Paragraph bridging Pages 8 and 9:

The invention will be described in greater detail with reference to the examples which are ~~provides~~provided to illustrate the invention. The examples are not to be construed to be limiting as to the scope of the invention, which is set forth in the appended claims.

Paragraph bridging Pages 9 and 10:

Due to the degeneracy of the DNA code, it will be well understood to one of ordinary skill in the art that substitution of nucleotides may be made without changing the amino acid sequence of the protein. Therefore, the invention includes any nucleic acid sequence for the human TREK-1 channel that encodes the amino acid sequence determined for ~~murine~~human TREK-1 (SEQ ID NO:2). Moreover, it is understood in the art that for a given protein's amino acid sequence, substitution of certain amino acids in the sequence can be made without significant effect on the function of the protein. Such substitutions are known in the art as "conservative substitutions." The invention encompasses human TREK-1 proteins that contain conservative substitutions, wherein the function of the protein is not altered. Generally, the identity of such ~~an~~a mutant TREK-1 will be at least 90% identical to SEQ ID NO:2. Preferably, the mutant TREK-1 will be at least 95% identical to SEQ ID NO:2. More preferably, the mutant TREK-1 will be at least 97% identical to SEQ ID NO:2. Most preferably, the mutant TREK-1 will be at least 99% identical to SEQ ID NO:2.

Page 10, first full paragraph:

The sequence for murine TREK-1, which is a corrected form of murine TREK-1 reported earlier, is shown in SEQ ID NO:4. There is a longer open reading frame than originally reported, producing a protein with a deduced amino acid sequence of 411 amino acids. Due to the degeneracy of the DNA code, it will be well understood to one of ordinary skill in the art that substitution of nucleotides may be made without changing the amino acid

sequence of the protein. Therefore, the invention includes any nucleic acid sequence for the murine TREK-1 channel that encodes the amino acid sequence determined for murine TREK-1 (SEQ ID NO:4). Moreover, as is the case with human TREK-1, it is understood in the art that for a given protein's amino acid sequence, substitution of certain amino acids in the sequence can be made without significant effect on the function of the protein. The invention encompasses murine TREK-1 proteins that contain conservative substitutions, wherein the function of the protein is not altered. Generally, the identity of such ~~an~~a mutant TREK-1 will be at least 90% identical to SEQ ID NO:4. Preferably, the mutant TREK-1 will be at least 95% identical to SEQ ID NO:4. More preferably, the mutant TREK-1 will be at least 97% identical to SEQ ID NO:4. Most preferably, the mutant TREK-1 will be at least 99% identical to SEQ ID NO:4.

Paragraph bridging Pages 12 and 13:

The actual concentrations of anesthetics were subsequently determined by means of a gas chromatography method (HP 6890 equipped with a DB624 column) using FID detection. ~~Sampled~~Samples (2.5 ml of solution) were collected prior to (t_0) and after perfusion (t_{45}) through the experimental setup. Solutions were collected using gas impermeable tubing and stored in sealed glass containers at 4°C for subsequent analysis. Samplings and measurements were performed in duplicate. Actual concentrations of anesthetics were determined by multiplying the calculated concentration by the ratio t_{45}/t_0 (chloroform: 0.16; halothane: 0.37, isoflurane: 0.76; and diethyl ether: 0.57). In the dose effect curves, the threshold concentrations were estimated as concentrations producing an increase higher than 10% in current amplitude.

Marked-Up Version Showing Changes Made to the Claims

(Amended) A method for identifying substances having anesthetic properties, wherein said substances produce a ~~safe,~~ reversible state of unconsciousness with concurrent amnesia and analgesia in a mammal upon inhalation comprising:

(a) contacting said substance with a mammalian potassium transport protein, wherein said potassium transport protein exhibits outward-going potassium rectification; and

(b) determining the potassium transport activity of said potassium transport protein, wherein an activation of potassium transport is indicative of said substance having anesthetic properties.

18. (Amended) A method for identifying substances having anesthetic properties, wherein said substances produce a ~~safe,~~ reversible state of unconsciousness with concurrent amnesia and analgesia in a mammal upon inhalation comprising:

(a) contacting said substance with COS cells, wherein said COS cells are transfected with a nucleotide vector comprising a nucleic acid molecule encoding TREK-1, wherein said COS cells transiently express said TREK-1 on a surface of said COS cells, and wherein <u>said</u> TREK-1 exhibits outward-going potassium rectification; and

(b) determining the potassium transport activity of said TREK-1 wherein an activation of potassium transport is indicative of said substance having said anesthetic properties.

19. (Amended) ~~The method of claim 18, wherein said TREK-1 comprises~~<u>A method for identifying substances having anesthetic properties, wherein said substances produce a reversible state of unconsciousness with concurrent amnesia and analgesia in a</u>

mammal upon inhalation comprising:

(a) contacting said substance with COS cells, wherein said COS cells are transfected with a nucleotide vector comprising a nucleic acid molecule encoding an amino acid sequence that is at least 95% identical to SEQ ID NO:2, wherein said COS cells transiently express said amino acid sequence on a surface of said COS cells, and wherein said amino acid sequence exhibits outward-going potassium rectification; and

(b) determining the potassium transport activity of said amino acid sequence wherein an activation of potassium transport is indicative of said substance having said anesthetic properties.

20. (Amended) ~~The method of claim 18, wherein said TREK-1 comprises~~A method for identifying substances having anesthetic properties, wherein said substances produce a reversible state of unconsciousness with concurrent amnesia and analgesia in a mammal upon inhalation comprising:

(a) contacting said substance with COS cells, wherein said COS cells are transfected with a nucleotide vector comprising a nucleic acid molecule encoding an amino acid sequence that is at least 95% identical to SEQ ID NO:4, wherein said COS cells transiently express said amino acid sequence on a surface of said COS cells, and wherein said amino acid sequence exhibits outward-going potassium rectification; and

(b) determining the potassium transport activity of said amino acid sequence wherein an activation of potassium transport is indicative of said substance having said anesthetic properties.

22. (Amended) A method for identifying substances having anesthetic properties,

wherein said substances produce a ~~safe,~~ reversible state of unconsciousness with concurrent amnesia and analgesia in a mammal upon inhalation comprising:

(a) contacting said substance with transfected cells, wherein said transfected cells are transfected with a nucleotide vector comprising a nucleic acid molecule encoding TASK, wherein said transfected cells transiently express said TASK on a surface of said transfected cells, and wherein said TASK exhibits outward-going potassium rectification; and

(b) determining the potassium transport activity of said TASK wherein an activation of potassium transport is indicative of said substance having said anesthetic properties.

23. (Amended) ~~The method of claim 22, wherein said TASK comprises~~A method for identifying substances having anesthetic properties, wherein said substances produce a reversible state of unconsciousness with concurrent amnesia and analgesia in a mammal upon inhalation comprising:

(a) contacting said substance with transfected cells, wherein said transfected cells are transfected with a nucleotide vector comprising a nucleic acid molecule encoding an amino acid sequence that is at least 95% identical to SEQ ID NO:5, wherein said transfected cells transiently express said amino acid sequence on a surface of said transfected cells, and wherein said amino acid sequence exhibits outward-going potassium rectification; and

(b) determining the potassium transport activity of said amino acid sequence wherein an activation of potassium transport is indicative of said substance having said anesthetic properties.

Please cancel Claims 17, 21 and 24 without prejudice and without disclaimer of the subject matter contained therein.

Remarks

We note with appreciation the rejoinder of Groups VI-XIV to elected Group V upon reconsideration of the Response dated July 9, 2001.

Claims 13-16, 18-20, and 22-25 are at issue in the case. Claims 17, 21, and 24 have been withdrawn without prejudice and without disclaimer of the subject matter contained therein.

Claims 19 and 23 have been amended into independent form to comply with 37 C.F.R. 1.75(c).

We respectfully submit that Claim 25 recites a proper Markush group. According to MPEP §2173.05(h),

> [w]hen the Markush group occurs in a claim reciting a process or a combination (not a single compound), it is sufficient if the members of the group are disclosed in the specification to possess at least one property in common which is mainly responsible for their function in the claimed relationship, and it is clear from their very nature or from the prior art that all of them possess this property.

As explained in the Specification at page 6, the cells expressing the potassium transport protein "may be of any type which can express the protein in appropriate conformation to allow for the transport of potassium." Each of the cell types listed in Claim 25 possess this common property. As such, we respectfully submit that Claim 25 is in proper form.

In regard to enablement, Claims 13-16, 18-20, and 22-25 have been broadened by eliminating the term "safe" therefrom. Furthermore, it is well-known in the art that conservative substitutions, for example, may be made in an amino acid sequence without altering the function of the protein. *See, e.g.*, Albert L. Lehninger et al., Principles of Biochemistry (Worth Publishers, 2d ed. 1993). In other words, one of ordinary skill in the art knows that replacement of an amino acid residue in a polypeptide by another residue with

similar properties, for example, replacement of glutamate by aspartate, is not likely to alter the function of the protein. Contrary to the assertion that "the specification has not taught how to modify the claimed variants of TREK and TASK in order to preserve the functional properties of the disclosed TREK and TASK," the Specification at page 7 explains that substantial identity is understood to mean that "amino acid substitutions may be made such that the overall conformation of the potassium transport protein is not significantly altered: the protein remains active as a potassium transport protein." Additionally, the Specification at page 9 states that "it is understood in the art that for a given protein's amino acid sequence, substitution of certain amino acids in the sequence can be made without significant effect on the function of the protein.... The invention encompasses ... proteins that contain conservative substitutions, wherein the function of the protein is not altered." Indeed, Claims 19, 20 and 23 are drawn only to those variants having 95% identity to SEQ ID Nos: 2, 4, and 5 which retain the function of the protein -- outward-going potassium rectification.

Of the amino acid substitutions set forth in the Official Action, only one conservative substitution reduced the biological activity of the protein. A single example does not support the contention that "what appears to be an inconsequential chemical modification will *often* dramatically affect the biological activity and characteristic of a protein." Moreover, one of ordinary skill in the art would expect nonconservative amino acid substitutions to affect the biological activity and characteristic of a protein. That the TWIK-1 and TREK-1 proteins share structural similarity but not functional similarity is fundamentally irrelevant since structural similarity refers to the overall conformation of the protein, not to the claimed 95% identity of the *primary* structure. We, therefore, respectfully submit that it is predictable that the claimed variants of SEQ ID Nos: 2, 4 and 5 will function in the claimed screening methods.

Turning to the prior art, we respectfully submit that Claims 13-16, 18, 22, and 25 are

patentably distinct over the cited hypothetical prior art combinations for the reasons detailed hereinafter.

A *prima facie* case of obviousness under 35 U.S.C. §103 has three requirements: there must be some suggestion or motivation to modify the reference or to combine the reference teachings; there must be a reasonable expectation of success; and the prior art references must teach or suggest all of the claim elements.

Claims 13-16, 18, 20, 22, and 25 are directed to method for identifying anesthetic substances by contacting a *mammalian* transport protein with the test substance and measuring the potassium transport activity thereof.

We first respectfully submit that no motivation to combine the Franks and Lieb article ("Franks") with either the Fink article ("Fink") or the Duprat article ("Duprat") has been established on this record. Franks teaches that an inhibitory synaptic K+ current, $I_{K(An)}$, found in molluscan neurons, "is rapidly and reversibly activated by anaesthetics, does not inactivate with time, is not voltage-gated, and responds stereoselectively to the optical isomers of isoflurane...." All of these properties accordingly make $I_{K(An)}$ "an attractive candidate for a target in general anaesthesia," assuming that $I_{K(An)}$ exists in mammals. Thus, Franks teaches only that $I_{K(An)}$ or its mammalian counterpart having *all* of the elucidated properties is a prime candidate as a target for general anesthesia. Furthermore, Franks does not present any teaching concerning the molecular target of the anesthetics.

In sharp contrast, Fink discloses the discovery of the TREK-1 protein. As taught therein, TREK-1 is a highly abundant, outwardly rectifying potassium channel. Duprat discloses the discovery of the TASK protein, also a highly abundant, outwardly rectifying potassium channel. There is no suggestion in either Fink or Duprat that the proteins disclosed therein are the mammalian counterparts to the $I_{K(An)}$ protein taught by Franks. Given the specificity of the teaching of Franks -- i.e., that $I_{K(An)}$ or its mammalian counterpart having all of the disclosed properties is a prime candidate for general anesthesia, the lack of

teaching or suggestion by both Fink and Duprat that the TREK-1 and TASK proteins are the mammalian counterparts to $I_{K(An)}$ or even possess the properties of $I_{K(An)}$ that make it a target for general anesthesia evidences the lack of motivation to combine Franks with either Fink or Duprat.

In short, as no motivation to combine the cited references has been established on this record, a *prima facie* case of obviousness has not been proven.

Moreover, even a hypothetical combination of Franks with Fink does not achieve the invention of Claims 16 and 20. Claims 16 and 20 encompass the methods for identifying anesthetic substances wherein the mammalian potassium transport protein has the 411 amino acid sequence of SEQ ID NO:4. In sharp contrast, the TREK-1 protein disclosed by Fink has only 370 amino acids. As noted in the Specification at page 10, SEQ ID NO:4 does not correspond to the sequence of murine TREK-1 disclosed previously. Accordingly, Claims 16 and 20 are patentable over any hypothetical combination of Franks and Fink.

Applicants have discovered a new and useful method for identifying substances having the specified anesthetic properties. In light of the foregoing, we respectfully submit that Claims 13-16, 18-20, and 22-25 are in proper form for allowance, which early action is hereby requested.

Respectfully submitted,

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